

TAXONOMIC VALUE OF CULM ANATOMICAL CHARACTERS IN THE SPECIES OF *BOUTELOUA* LAGASCA (POACEAE: ERAGROSTIDEAE)

Maria Elena Siqueiros-Delgado

Centro básico, Universidad Autónoma de Aguascalientes, Avenida Universidad s/n,
Aguascalientes, Ags., 20100, MEXICO

Yolanda Herrera-Arrieta

CIIDIR Unidad Durango, Instituto Politécnico Nacional, Apartado Postal 57,
Durango, Dgo., 34000, MEXICO
Becaria de la COFAA

ABSTRACT

The study of anatomical characters in the culm epinodal zone of 41 species and sixteen varieties of *Bouteloua* has been completed. Worthless taxonomic characters were found in this region, nevertheless, five anatomical patterns were characterized. Other regions of the culm were explored for a possible later study.

KEY WORDS: *Bouteloua*, Poaceae, anatomy, systematics

RESUMEN

Se llevó a cabo un estudio de los caracteres anatómicos en la zona epinodal del tallo de 41 especies y dieciséis variedades del género *Bouteloua*. Se encontraron caracteres de escaso valor taxonómico en esta región del tallo, no obstante, se caracterizaron cinco patrones anatómicos diferentes. Se complementó el estudio con observaciones en otras regiones del tallo, para un posible estudio posterior.

PALABRAS CLAVE: *Bouteloua*, Poaceae, anatomía, sistemática

INTRODUCTION

Systematic studies in plants are becoming more important up to date. From their results we can get a knowledge closer to the reality about evolution of the plants and their phylogenetic relationships, particularly in those species with any kind of importance. This is the case of the *Bouteloua* species, which are some of the most important native grasses in North American grasslands because of their great forage quality, their resistance to heavy livestock, and wide distribution. Species of *Bouteloua* were once the dominant components in the Mexican grasslands at the beginning of this century. However, in spite of being considered the producers of high quality beef in free-ranging livestock, their biology and taxonomy are not well defined until now, and the confusion in species definition remains. This genus has been studied totally or partially by: Griffiths (1912); Hitchcock (1920, 1935, 1939); Swallen (1939); Gould (1968, 1969, 1975, 1979); Gould & Kapadia (1964), and Roy (1968). All those studies were morphological taxonomic studies more than systematic ones. Gould, *et al.* incorporated chromosomal information in addition to morphological data, but no other kinds of features have been studied in this genus in order to get an exact inference related to the evolution of its taxa. Columbus (1996) studied in his Ph.D. research, some aspects of a systematic study in this genus, together with other related genera. The topic of his research was the anatomy of leaf sections and molecular DNA in *Bouteloua* and genera related. From these two features he reached a conclusion about the phylogeny of the tribe Boutelouineae in America. In other studies, Herrera and colleagues began research to study basic features such as anatomy in leaf epidermis and culm sections, flavonoid profiles, and physiographic requirements for the development of the Mexican taxa.

The present study is part of an ongoing project "A biosystematic study of the genus *Bouteloua* from México." At the same time it pretends to contribute to the Columbus research of *Bouteloua* from America. The particular objective of this project is to characterize worthy anatomical features from culm sections in the taxa of *Bouteloua*, in order to add data to support the better understanding of its phylogenetic relationships.

It is widely known that anatomical studies in leaf sections add to the general knowledge of the plants with good results (Ellis 1976; Columbus 1996; Herrera & Grant 1994; Aiken, *et al.* 1984). However, there are few studies of culm anatomy, none in *Bouteloua*. Authors like Sánchez (1983a, 1983b), Ebinger & Carlen (1975), Auquier & Somers (1967), and De Wet (1960) have generated anatomical data from culm sections in several grass genera other than *Bouteloua*. All these authors agree about the phylogenetic value of culm anatomy, in addition to other features at the supraspecific level.

MATERIALS AND METHODS

The culm sections studied here were made in the proximal node area (epinodal zone) in 41 species and sixteen varieties of *Bouteloua* from America, all of them from fresh collections done by Columbus (1996) (all the taxa with numbers in this work.

e.g., *B. alamosana* [2418]) in México and other countries in America. Complementary fresh material collected by Herrera and colleagues, as well as herbarium specimens of rare species were used. Three hundred permanent slides with four to six repetitions per species, were done at the Anatomy Laboratory of Rancho Santa Ana Botanic Garden, Claremont, Calif. The sections were cut at exactly the basal portion of the central internode of well developed culms, then processed as follows:

Desilicification: 30% Hydrochloric acid was used for 48 hrs to remove silica from culms.

Dehydration: was in several consecutive steps of 1 hr each, in ethanol at different concentrations (30%, 50%, 70%, 90%), then 100% + 1% safranin to dye the tissues and to facilitate the inclusion and sectioning, then 100% ethanol alone.

Clearing: consecutive steps 1 hr each were done in the following solutions: 2:1 ethanol: xylene, 1:2 ethanol: xylene, 100% xylene twice.

Impregnation: 1:1 Xylene: paraffin oil, 2 hrs; Paraffin at 58° C, 6 hrs, twice.

Inclusion: was done in a special device to include tissue in the position required to make slices.

Paraffin blocks with the tissue included were placed in a softener (10% aerosol OT: glycerine: water 10:3:90) from Schmid & Turner (1977), during two days in order to facilitate slicing. Blocks then were placed on ice at least 1 hr, then sliced with a Spencer 820 microtome. Slices of 10 µm thickness were cut, then dyed following the Sharman (1943) procedure. Finally, the analysis and description of the anatomical characters was done.

To get a comparative picture of the culm in *Bouteloua*, other regions than the epinodal were observed; these included the nodal zone and the central part of the internode. Fresh material from specimens collected in several parts of México by the collaborators of this project were used to analyze the differences and to validate the characters from other culm levels.

RESULTS

The analysis of the fixed slices and the fresh slices permitted observation of the anatomy of the epinodal zone, as well as the nodal and central part of the internode (Figure 1), where it is possible to find several kinds of cells, organized in different ways, forming defined patterns depending primarily on the maturity and level of the culm. The main types of cells found are:

epidermal cells (Figure 2.1), associated with silica cells, trichomes and stomata;

parenchyma cells, thin walled large cells which can form a medullar parenchyma (Figure 2.2), a subepidermal ring in the nodal region, or radial rachimorph parenchyma cells (chlorenchyma) which are responsible for culm photosynthesis (Figure 2.3), always placed perpendicularly to the epidermal surface and associated with the Kranz cells;

sclerenchyma cells (Figure 2.4), thick walled cells with a small lumen forming subepidermal rings and generally surrounded by the vascular bundles, making them more conspicuous at the apex;

intermediate cells (Figure 4.1), morphologically intermediate cells which share features from both parenchyma and sclerenchyma cells, very abundant in the culm and difficult to define, they form subepidermal rings in proximal parts of the node, or they can be mixed in the sclerenchyma ring. Some authors mention collenchyma cells in the culm, they are probably referring to this type of cells (Sánchez 1981);

mestome cells (Figure 6.4), thick walled cells that completely surround the peripheric vascular bundles and the interior ones, they can be variable in size, number, and shape, and occasionally they look like sclerenchyma cells;

vascular bundles, can be internal (IVB, Figure 3.1) or peripheral (PVB, Figure 3.2), the former are in the interior part of the culm and are primary vascular bundles (Figure 3.3), occasionally secondary vascular bundles (Figure 3.4), while the later (PVB) can be secondary or even tertiary vascular bundles (Figure 3.5). Generally in well differentiated culm zones, the PVB are tertiary and are associated with Kranz cells and photosynthetic parenchyma, while in the immature zones the PVB are secondary vascular bundles;

Kranz cells (Figure 3.6), large conspicuous cells, thin walled, associated with tertiary vascular bundles, in the exposed mature zones of the internodal sheath. These cells together with the radial rachymorph chlorenchyma form a system called Kranz syndrome (Sánchez 1979), this system (Figure 3.7) is associated with the C₄ photosynthetic process.

Anatomical description of the culm internode at several levels

Epinodal zone. Analysis of epinodal zone slides showed a rounded to oval transection, with smooth to lightly undulating outline. This culm zone of *Bouteloua* resulted in a very variable region for anatomical characters, however, it was possible to observe five well defined patterns in this zone, which are variable if the level of section is moved within just a few millimeters.

1. Subepidermal ring of intermediate cells without sclerenchyma.

The subepidermal ring of intermediate cells which bound the PVB, these generally are secondary VB, in some species showing an incipient Kranz system, the IVB are in one or several levels and can be primary or secondary VB. The number and size of the VB is variable among the species. Species containing this pattern are: *Bouteloua alamosana* [2418], *B. aristidoides* var. *aristidoides* [2133, 2217], *B. disticha* [2376], *B. gracilis* [2223], *B. media* [2367, 2420], and *B. rigidiseta* [2231] (Figure 4).

2. Subepidermal ring of intermediate cells bounding a sclerenchyma ring.

The subepidermal ring of intermediate cells show the internal margin undulated, with 3 to 4 cells of thickness in the widest part, and two in the narrower. This ring surrounds a sclerenchyma ring of two cells thickness that joins the VB, the PVB are totally included, they generally are II°, and bound the external portion the IVB can be primary or secondary. An incipient Kranz system is present in some samples. Species with this pattern are: *Bouteloua alamosana* [2301], *B. barbata* var. *barbata* [2268], *B. chondrosioides* [2422], *B. curtispindula* var. *caespitosa* [2213, 2326], *B. disticha* [2393], *B. elata* [2366], *B. eriostachya* [2286], *B. media* [2367, 2387], *B.*

chihuahuana [2289], *B. rigidiseta* [2231, 2233], *B. simplex* [2278], *B. triaena* [2338, 2357], and, *B. williamsii* [2419] (Figure 5).

3. Subepidermal ring of sclerenchyma alternated with intermediate cells. The subepidermal ring of sclerenchyma is variable in thickness, it surrounds the PVB and borders the external margin of the IVB, alternating with irregular zones of intermediate cells in some slides, where the ring is interrupted by Kranz cells with associated radial parenchyma. Species presenting this pattern are: *Bouteloua annua* [2433], *B. aristoides* var. *aristoides* [2359], *B. barbata* var. *barbata* [2229, 2268], *B. disticha* [2360], *B. elata* [358], *B. eludens* [2274], *B. eriopoda* [2127, 2214], *B. gracilis* [2340], *B. hirsuta* [2342], *B. parryi* var. *parryi* [2351], *B. reflexa* [2436], *B. scorpioides* [2200], *B. simplex* [2335], *B. trifida* [2126], and *B. williamsii* [2353] (Figure 6).

4. Ring of intermediate cells of sclerenchyma only in the top of vascular bundles. The ring of intermediate cells totally surrounds the PVB (generally 11°) with 3 to 6 thicknesses of cells, and borders the IVB only in the top. The sclerenchyma cells surround the VB in the top. Species presenting this pattern are: *Bouteloua annua* [2434], *B. hirsuta* var. *hirsuta* [2284], *B. megapota mica* [28393], *B. parryi* var. *gentryi* [2369], *B. repens* [2123], and *B. vaneedenii* [2542] (Figure 7).

5. Culm with well defined Kranz system. The sclerenchyma ring of variable thickness, interrupted by a variable number of Kranz cells like a crown, radial parenchyma is always associated, IVB in one or many levels. Species presenting this pattern are: *Bouteloua aristoides* var. *aristoides* [2217], *B. barbata* var. *rothrockii* [2215], *B. curtispindula* var. *tenuis* [2352], *B. pedicellata* [2408], *B. ramosa* [2287], *B. repens* [2350, 2396, 2425], and *B. triaena* [2401] (Figure 2).

Observations done at nodal and internodal zones from fresh sections of some *Bouteloua* species resulted as follows:

Nodal zone. The nodal culm region presents a circular and smooth transection with a subepidermal variable thickness parenchyma ring surrounding a thick sclerenchyma ring. The sclerenchyma ring links the VB's which are very close to each other. An unclear definition of the parenchyma ring and the VB's is found in the initial node zone; sheath and culm are not well differentiated in this particular zone, appearing like a unit (Figure 8). This pattern is present in complete sequences of *Bouteloua breviseta* [2279] and *B. rigidiseta* var. *rigidiseta* [2231]. A different pattern in the same section level with external and internal sclerenchyma rings was found in two other species: *B. karwinskii* [2208] and *B. johnstonii* [2285], where two rings of sclerenchyma are present, one external surrounding the parenchyma ring and another internal linking the undefined VB's.

Examined species: *Bouteloua aristoides* var. *aristoides* [2279], *B. barbata* var. *barbata* [2318], *B. breviseta* [2279], *B. chasei* [2197], *B. chondrosioides* [2184], *B. curtispindula* var. *caespitosa* [2177], *B. eludens* [2272], *B. gracilis* [2201], *B. hirsuta* [2216], *B. karwinskii* [2208], *B. media* [2367, 2387], *B. pectinata* [2823], *B. purpurea* [2343], *B. radicata* [2318], *B. ramosa* [2137], *B. rigidiseta* var. *rigidiseta* [2231], *B. scorpioides* [2320], and *B. uniflora* [2319].

Mid internodal zone. According to the observations from fresh or herbarium material slices, the anatomical pattern in this culm level (always devoid of sheath), seems to be constant in all the superior half of the internodal zone, it matches with the Auquier & Somers (1967) description of a chloridoid type. There exist few variations depending on the species, mainly related to the number and position of the VB's, and the sclerenchyma ring thickness. The observations made in this zone were aimed at establishing differences among the anatomical features from the epinodal and the internodal zones, as well as to find important systematic characters. The description of this pattern is shown as follows (Figure 3). Transections oval to round with smooth to undulate outline, epidermis with square or round cells, few trichomes and stomata opening to the radial chlorenchyma. The subepidermal zone shows a continued sclerenchyma ring, interrupted by the Kranz system, this ring consists of 3 to 8 cells in thickness, surrounding the PVB, and bounding only the IVB top margin. The Kranz system with 4 to 8 crown shaped cells on the radial parenchyma, associated with the III° VB; sclerenchyma girder associated with the PVB; VB's variable in number and position according to the species, PVB's in this zone are mostly III° with clearly associated Kranz system; IVB's can be distributed in one or several levels, they generally are I°, sometimes II°. The IVB's are always surrounded by their mestome sheath, which can have a single or double layer at the bottom.

Examined species: *Bouteloua alanosana* [Herrera & Siqueiros 1319] (CIIDIR), *B. aristidoides* var. *aristidoides* [Rosales s/n] (HUAA), *B. barbata* var. *barbata* [Siqueiros s/n] (HUAA), *B. barbata* var. *sonorae* [Herrera & Siqueiros 1309] (CIIDIR), *B. chondrosioides* [Herrera 1402] (CIIDIR), *B. curtispindula* [Herrera 1324] (CIIDIR), *B. elata* [Herrera 1465] (CIIDIR), *B. parryi* [Herrera & Siqueiros 1315] (CIIDIR), *B. pedicellata* [Rzedowski 44714] (IEB), *B. quiriegoensis* [Herrera & Siqueiros 1320] (CIIDIR), *B. radicata* [Herrera 1469] (CIIDIR), *B. reflexa* [Herrera & Siqueiros 1311] (CIIDIR), *B. uniflora* [Herrera & Siqueiros 1307] (CIIDIR), and *B. williamsii* [Herrera 1300] (CIIDIR).

DISCUSSION

The study of the *Bouteloua* culm anatomy resulted in two main problems: the enormous anatomical variation at different levels, and the confusion and lower definition of some tissue cells. Metcalfe (1960) suggested that culm anatomy has a limited taxonomic value, because of the variation at different levels, a theory which has been corroborated by the results of this work, in the epinodal part of the *Bouteloua* culm.

Anatomical features in the epinodal zone cannot be considered as key characters to add in the delimitation of *Bouteloua* species, neither in the determination of phylogenetic position; since it is a culm menistematic region, with different patterns at different levels. The five more stable patterns described in the results were based on the presence or absence and position of the sclerenchyma cells, as well as on the position of the ring of intermediate cells. The general observance for all the species is

the presence of immature tissues (not completely differentiated) and a ring of intermediate cells bordering the VB's, which is replaced by sclerenchyma cells far away from the node, Kranz cells are not present in this region. The VB's seem to be very mature in this zone, without variation from one region to another. It is difficult to establish a specific pattern inside the populations of one species, they commonly present intermediate schemes. The task of trying to assemble taxa related by anatomical features, and to compare them with phylogenetic groups previously established by Columbus (1996), did not succeed. Different taxa having the same pattern of culm anatomy do not represent close relationship (morphological, DNA content or leaf anatomy features). Unexpectedly, more than one culm anatomic pattern may be found inside populations of a single species, e.g., *B. alamosana* [2418] shows pattern # 1, while [2301] shows pattern # 2; *B. disticha* [2393] has pattern # 2, and [2360] has pattern # 3. This is probably due to millimetric differences in the level of cutting.

Unexpectedly, the nodal zone was less variable, showing a specific anatomic pattern, which is easy to distinguish because it is very diffuse and the VB's are very close to each other, and formed a continuous ring of sclerenchyma which surrounds the entire VB. A subepidermal ring of parenchyma is often present, it rarely shows two sclerenchyma rings as observed in *Bouteloua karwinskii* and *B. johnstonii*. This nodal pattern changed towards upper levels, firstly substituting the typical parenchyma ring of this region for a ring of intermediate cells with a different arrangement (as commented above); then, in a higher level, this ring of intermediate cells was changed for sclerenchyma cells and the Kranz cells started showing up. The first cells appeared laterally placed, one on each side of the PVB, then two on each side, and so on until they completed a crown in the apex of the PVB. The complete crown was present at the uncovered zone of the sheath which corresponds to the central part of the internode. Kranz cells were always associated with the radial parenchyma, which depending on the species can be conspicuous to inconspicuous. The presence of a mature Kranz system in this part of the culm, was probably due to the direct solar exposure, since it was devoid of the sheath protection at this portion, as Sánchez (1979, 1981) stated. At this culm level the resulting pattern remained constant in the reviewed populations.

It is necessary to underline that the present study was based on the epinodal culm features, whose permanent slides were all done. The observations from higher levels of the internode were done with fresh material, so that the quality was much lower, and no key characters were determined, nevertheless it was possible to make general descriptions of the principal patterns. The number of repetitions per species was not enough to make generalizations, however, it was possible to get a precise idea of the culm anatomy in this particular region. It is obviously necessary to make a future study of the anatomical variation at the central part of the culm internode.

In spite of the good fixed slides obtained in this work, sometimes it was impossible to define the cells corresponding to the kind of tissue in observation, especially in the epinodal zone, due to the intermediate cells mentioned and described above, which are morphologically intermediate between parenchyma and sclerenchyma cells, e.g., the cell wall thickness and the size. These cells have been catalogued by other authors as collenchyma cells (Sánchez 1984; De Wet 1960), or as tissue in transition or parenchyma in transition (Sánchez 1979, 1983a, and 1983b). Stevenson & Mertens (1980) stated that the collenchyma and the parenchyma are physically alike,

since they both are bearing living and active protoplasts surrounded by primary walls, differing in the wall thickness and the length of the cells. The close relationship between these two tissues is very evident in the culms. Esau (1972) stated that in the adjacent region of collenchyma and parenchyma, there exist cells in transition, which share features from both cell types. Metcalfe (1960), mentions that there is frequently a transition between thin walled parenchymatous cells and thicker walled prosenchyma (sclerenchyma) cells. This transition can be gradual or abrupt and sometimes it is difficult to define the kind of cells under observation. Tissue in transition or intermediate cells are more common in the undifferentiated culm regions, where it is possible to find this tissue forming subepidermal rings, continuous or alternating with other tissues like sclerenchyma. When the alternating tissue is parenchyma, it is difficult to define its boundaries. The parenchyma cells from the culm anatomy are clearly defined as a radial parenchyma associated with the Kranz structure and to the medulla cells when present.

During this exhaustive review of culm anatomical characters, searching for similarities and dissimilarities to define the taxonomic relationships, quite variable features were found at intra and interspecific level, e.g., the position of the ring of intermediate cells is not constant in the same individual at different culm levels; and also in different species. On the other hand, some features remain constant in the same species, but with minor variation between different species; that is the case of the VB's which present very low ranges of interspecific variation.

Lastly, it is important to remark that the information generated by this study will be taken as part of the biosystematic study of the genus, and even though the initial hypothesis was not corroborated, the data generated are worthy, and contributes to the general knowledge of the genus *Bouteloua*.

CONCLUSIONS

The main conclusion from this work is that the anatomic features from the epinodal culm zone in the taxa of *Bouteloua*, cannot be considered good systematic characters, because of the extreme variation presented. However, the central internodal zone seems to be much more constant, unless additional studies suggest different conclusions.

ACKNOWLEDGMENTS

This work was partially supported by CONABIO (B061) and CONACyT (3098-N). Special thanks are given to Travis Columbus for his kind advice and all the facilities to work with his freshly collected material, at his laboratory in Rancho Santa Ana Botanic Garden.

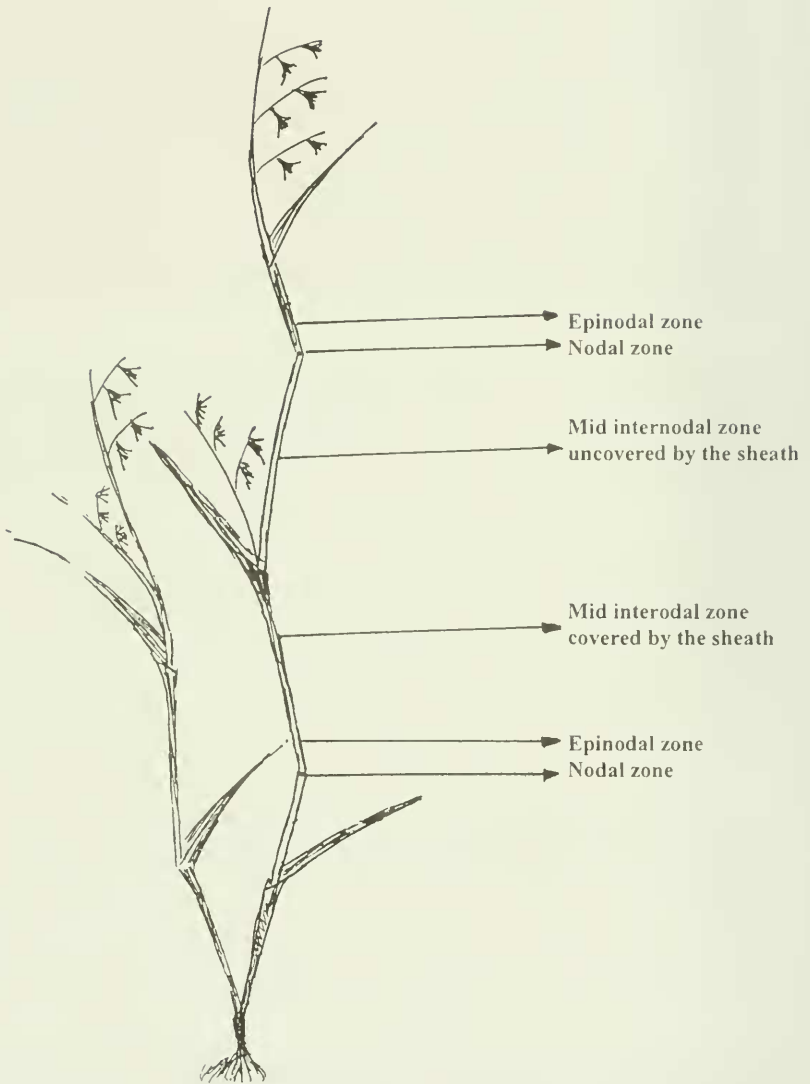


Fig. 1. Levels of culm sliced to observe: Nodal zone, Epinodal zone, Mid internodal zone

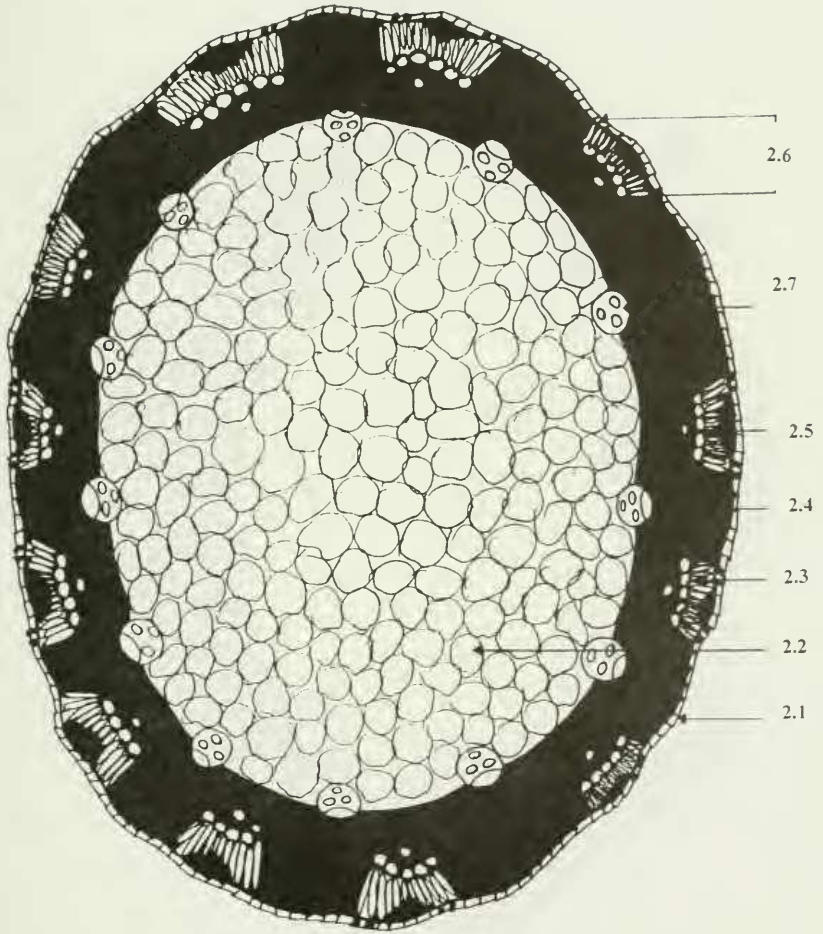


Fig. 2. *Bouteloua uniflora* Vasey. Culm transversal section at the mid internodal zone. 2.1) Epidermis. 2.2) Medullar parenchyma cells. 2.3) Radial parenchyma cells. 2.4) Sclerenchyma. 2.5) Vascular bundles. 2.6) Kranz system. 2.7) Internal vascular bundles.

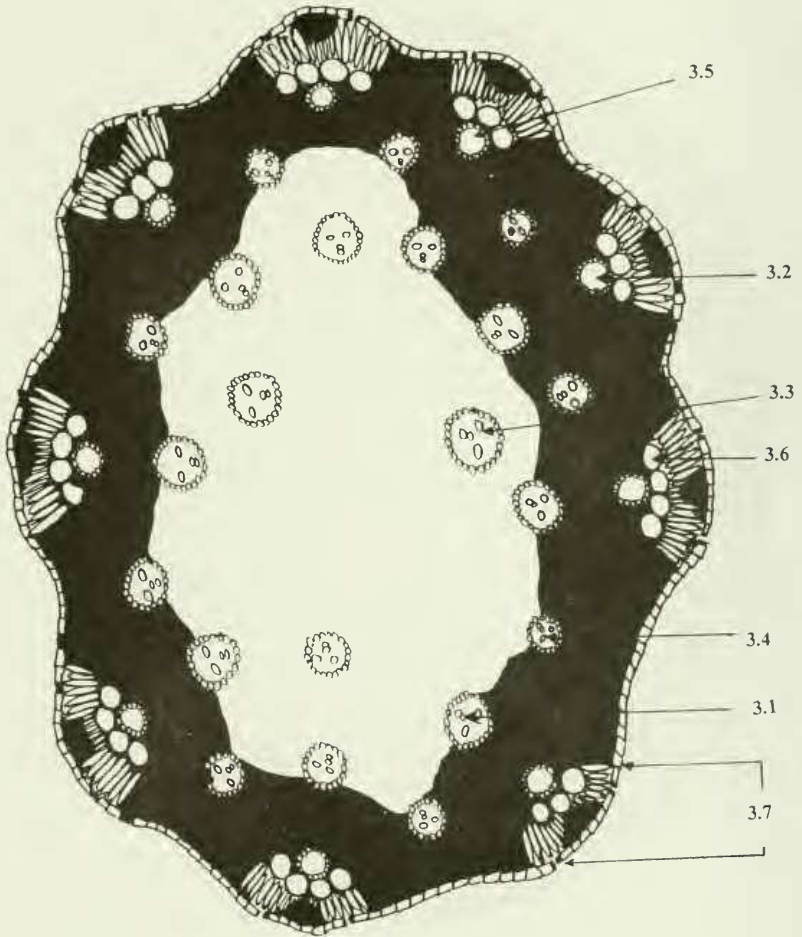


Fig. 3. *Bouteloua pedicellata* Swallen. Culm transversal section at the mid internodal zone. 3.1) Internal vascular bundles (IVB). 3.2) Peripheral vascular bundles. 3.3) Primary vascular bundles. 3.4) Secondary vascular bundles. 3.5) Tertiary vascular bundles. 3.6) Kranz cells. 3.7) Kranz system.

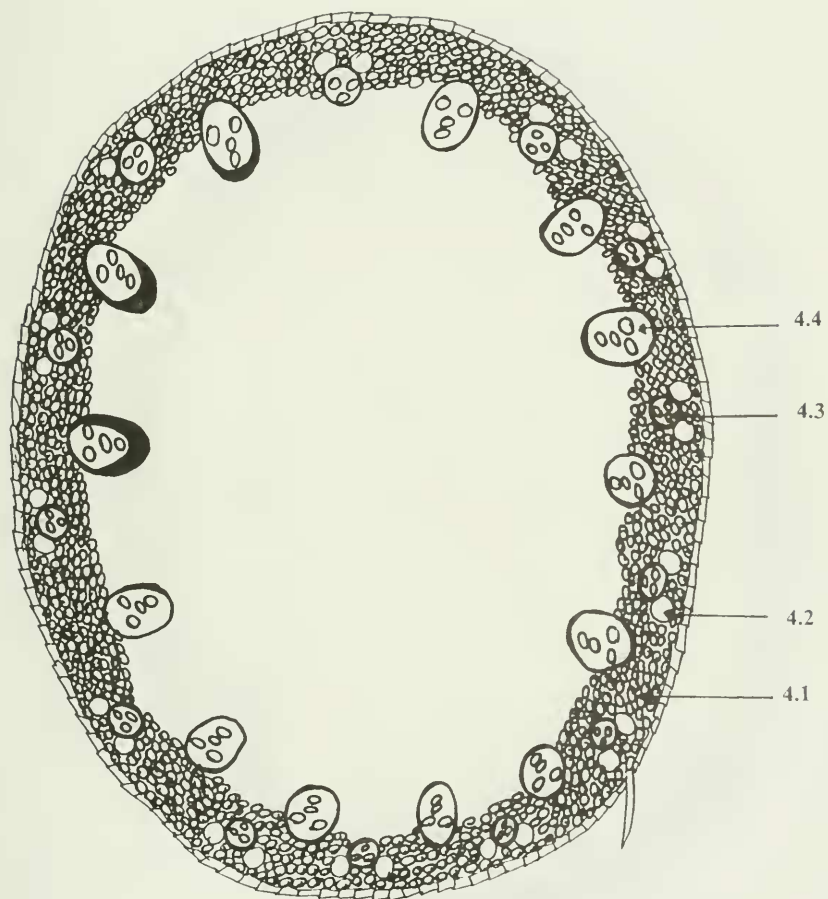


Fig. 4 *Bouteloua alamosana* Vasey. Culm transversal section at the epinodal zone. Subepidermal ring of intermediate cells without sclerenchyma. 4.1) Intermediate cells. 4.2) Kranz cells. 4.3) Secondary peripheral vascular bundles. 4.4) Internal vascular bundles.

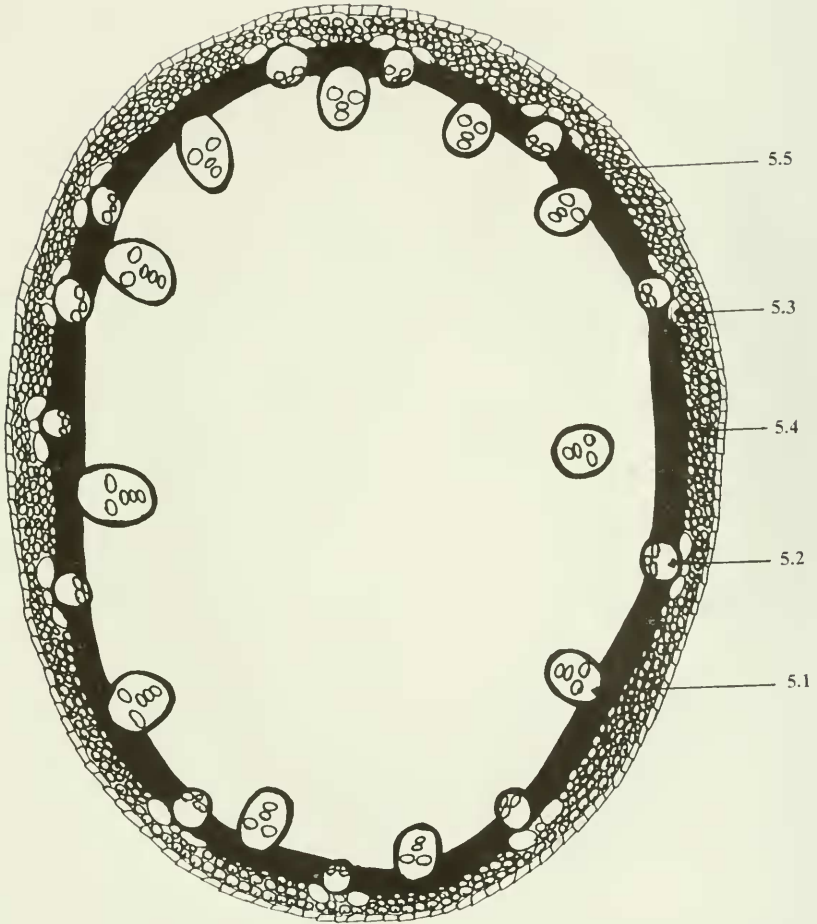


Fig. 5. *Bouteloua alamosana* Vasey. Culm transversal section at the epinodal zone. Subepidermal ring of intermediate cells surrounding a ring of sclerenchyma. 5.1) Primary vascular bundles. 5.2) Secondary vascular bundles. 5.3) Kranz cells. 5.4) Ring of intermediate cells. 5.5) Ring of sclerenchyma.

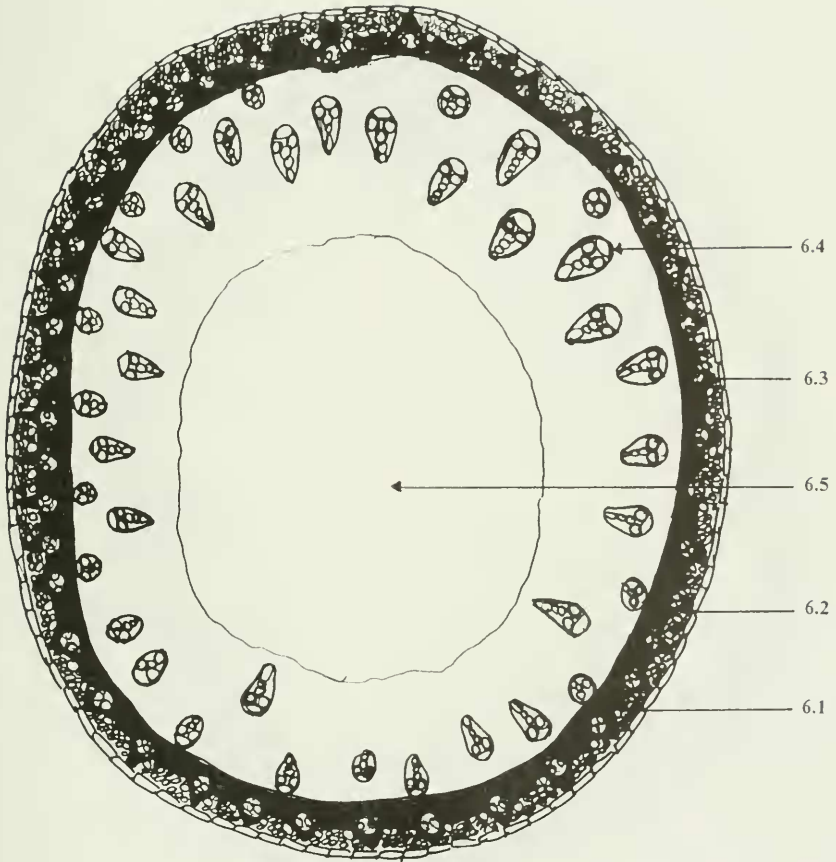


Fig. 6. *Bouteloua elata* Reeder & Reeder. Culm transversal section at the epinodal zone. Subepidermal ring of sclerenchyma alternating intermediate cells. 6.1) Intermediate cells. 6.2) Ring of sclerenchyma 6.3) Peripheral vascular bundles. 6.4) Internal vascular bundles. 6.5) Medulla.

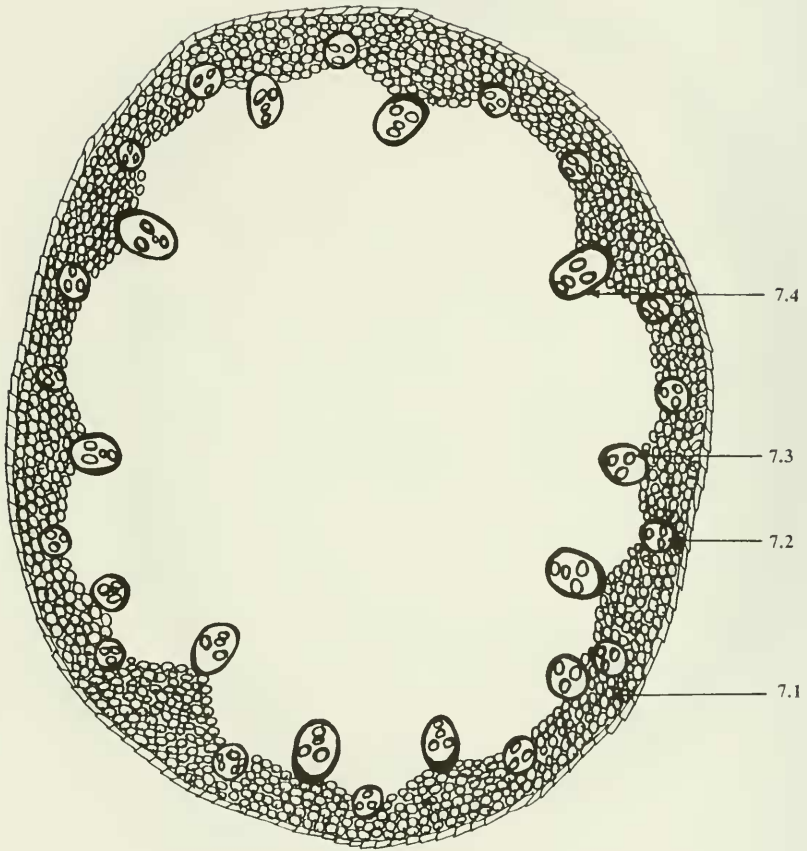


Fig. 7. *Bouteloua vaneedenii* Pi'ger ex Urban. Culm transversal section at the epinodal zone. Ring of intermediate cells with sclerenchyma only at the apex of the vascular bundles. 7.1) Intermediate cells. 7.2) Secondary peripheral vascular bundles. 7.3) Internal vascular bundles. 7.4) Sclerenchyma.

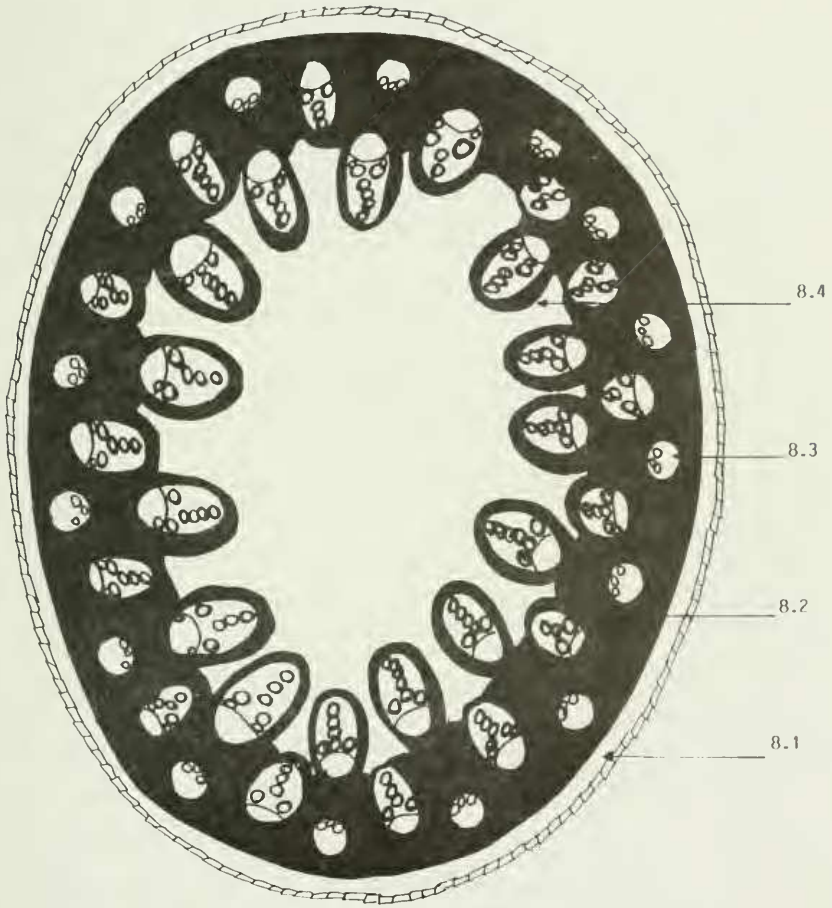


Fig. 8. *Bouteloua radicata* (Fournier) Griffiths. Culm transversal section at the nodal zone. 8.1) Ring of parenchyma. 8.2) Ring of sclerenchyma. 8.3) Secondary peripheral vascular bundles. 8.4) Internal vascular bundles.

LITERATURE CITED

- Aiken, S.G., S.J. Darbyshire, & L.P. Lefkovitch. 1984. Restricted taxonomic value of leaf sections in Canadian narrow leaved *Festuca* (Poaceae). *Can. J. Bot.* 63:995.
- Auquier, P. & Y. Somers. 1967. Recherches Histotaxonomiques sur le Chaume des Poaceae. *Bull. Societe Royale Botanique de Belgique*. Tome 100, p. 95.
- Columbus, J.T. 1996. Lemma Micromorphology, Leaf Blade Anatomy, and Phylogenetics of *Bouteloua*, *Hilaria* and relatives (Gramineae: Chloridoideae: Boutelouinae). Ph.D. Dissertation. University of California, Berkeley, California.
- De Wet, J.M. 1960. Culm anatomy in relation to taxonomy. *Bothalia* 7:311.
- Ebinger, J.E. & J.L. Carlen. 1975. Culm morphology and grass systematics. *Trans. Illinois State Acad. Sci.* 68:2.
- Ellis, R.P. 1976. A procedure for standardizing comparative leaf anatomy in the Poaceae. I. The leaf-blade as viewed in transverse section. *Bothalia* 12:65.
- Esau, K. 1972. *Anatomía Vegetal*. Ediciones Omega. 2a Ed. Barcelona, Spain.
- Gould, F.W. 1968. *Grass Systematics*. Mc Graw-Hill, New York, New York. 382 pp.
- Gould, F.W. 1969. Taxonomy of the *Bouteloua repens* complex. *Brittonia* 21:261.
- Gould, F.W. 1975. *The Grasses of Texas*. Texas A & M University Press, College Station, Texas. 653 pp.
- Gould, F.W. 1979. The genus *Bouteloua* (Poaceae). *Ann. Missouri Bot. Gard.* 66:384.
- Gould, F.W. & Z.J. Kapadia. 1964. Biosystematic studies in the *Bouteloua curtipendula* complex. II. Taxonomy. *Brittonia* 16:182.
- Griffiths, D. 1912. The Grama Grasses: *Bouteloua* and related genera. *Contr. U.S. Natl. Herb.* 14:343.
- Herrera-Arrieta, Y. & W.F. Grant. 1994. Anatomy of the *Muhlenbergia montana* complex. *Amer. J. Bot.*
- Hitchcock, A.S. 1920. The genera of Grasses of the United States with special Reference to the Economic Species. U.S.D.A. Bull. 772.1.
- Hitchcock, A.S. 1935. *Manual of the Grasses of the United States*. U.S.D.A. Misc. Publ. 200:1.
- Hitchcock, A.S., J.R. Swallen, & A. Chase. 1939. Grasses: North American Flora. 17(8):543.
- Metcalf, C.R. 1960. *Anatomy of the Monocotyledons*. I. Gramineae. Clarendon Press, Oxford, Great Britain. 731 pp.
- Roy, G.P. 1968. A Systematic Study of the *Bouteloua hirsuta* - *Bouteloua pectinata* complex. Ph.D. Dissertation. Texas A & M University, College Station, Texas.
- Sánchez, E. 1979. Estructura Kranz en tallos de Gramineae, (Eragrosteae). *Kutziana* 12-13:113.
- Sánchez, E. 1981. Desarrollo de la Estructura Kranz en tallos de Gramineae. *Lilloa* 35, 3:37.
- Sánchez, E. 1983a. Estudios Anatómicos en *Blepharidachne* Hackel (Poaceae, Eragrostideae, Eragrosteae). *Rev. Museo Arg. Cs. Nat. "Bernardino Rivadavia"* 6(3):73.
- Sánchez, E. 1983b. *Dasyochloa* Willdenow ex Rydberg (Poaceae). Género monotípico de Norteamérica. *Lilloa* 36:131.

- Sánchez, E. 1984. Estudios anatómicos del género *Munroa* (Poaceae, Chloridoideae, Eragrostidae). *Darwiniana* 25(1-4):43.
- Schmid, R. & M.D. Turner. 1977. Contrad 70, an effective softener of herbarium material for anatomical study. *Taxon* 26:551.
- Sharman, B.C. 1943. Tannic acid and iron alum with safranin and orange G in studies of the shoot apex. *Stain Technol.* 3:105.
- Stevenson, F.F. & T. Mertens. 1980. *Anatomía Vegetal*. Ed. Limusa. 1a. ed. México City, Mexico.
- Swallen, J.R. 1939. Chloridae. In *North American Flora*. New York Botanical Garden. Bronx, New York. 17:579.